

WHAT IS CLAIMED IS:

1. A fibronectin type III (Fn3) molecule, wherein the Fn3 comprises a stabilizing mutation as compared to a wild-type Fn3.
2. The Fn3 of claim 1, wherein the stabilizing mutation comprises at least one aspartic acid (Asp) residue that has been deleted or substituted with at least one other amino acid residue.
3. The Fn3 of claim 2, wherein Asp 7 or Asp 23, or both, have been deleted or substituted with at least one other amino acid residue. *count*
4. The Fn3 of claim 3, wherein Asp 7 or Asp 23, or both, have been substituted with an asparagine (Asn) or lysine (Lys) residue.
5. The Fn3 of claim 1, wherein the stabilizing mutation comprises at least one glutamic acid (Glu) residue that has been deleted or substituted with at least one other amino acid residue.
6. The Fn3 of claim 5, wherein Glu 9 has been deleted or substituted with at least one other amino acid residue.
7. The Fn3 of claim 6, wherein Glu 9 has been substituted with an asparagine (Asn) or lysine (Lys) residue.
8. The Fn3 of claim 2, wherein Asp 7, Asp 23, and Glu 9 have been deleted or substituted with at least one other amino acid residue.
9. A fibronectin type III (Fn3) polypeptide monobody comprising a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences,
wherein one or more of the monobody loop region sequences vary

by deletion, insertion or replacement of at least two amino acids from the corresponding loop region sequences in wild-type Fn3;

wherein the β -strand domains of the monobody have at least a 50% total amino acid sequence homology to the corresponding amino acid sequence of wild-type Fn3's β -strand domain sequences; and

wherein the Fn3 comprises a stabilizing mutation.

- Fn3 Cont'd*
10. An isolated nucleic acid molecule encoding the Fn3 molecule of claim 9.
 11. An expression vector comprising an expression cassette operably linked to the nucleic acid molecule of claim 10.
 12. A host cell comprising the vector of claim 11.
 13. The monobody of claim 9, wherein at least one loop region is capable of binding to a specific binding partner (SBP) to form a polypeptide:SBP complex having a dissociation constant of less than 10^{-6} moles/liter.
 14. The monobody of claim 9, wherein at least one loop region is capable of catalyzing a chemical reaction with a catalyzed rate constant (k_{cat}) and an uncatalyzed rate constant (k_{uncat}) such that the ratio of k_{cat}/k_{uncat} is greater than 10.
 15. The monobody of claim 9, wherein one or more of the loop regions comprise amino acid residues:
 - i) from 15 to 16 inclusive in an AB loop;
 - ii) from 22 to 30 inclusive in a BC loop;
 - iii) from 39 to 45 inclusive in a CD loop;
 - iv) from 51 to 55 inclusive in a DE loop;
 - v) from 60 to 66 inclusive in an EF loop; and
 - vi) from 76 to 87 inclusive in an FG loop.

- (b) Fn3 Cont.
16. The monobody of claim 9, wherein the monobody loop region sequences vary from the wild-type Fn3 loop region sequences by the deletion or replacement of at least 2 amino acids.
 17. The monobody of claim 9, wherein the monobody loop region sequences vary from the wild-type Fn3 loop region sequences by the insertion of from 3 to 25 amino acids.
 18. An isolated nucleic acid molecule encoding the polypeptide monobody of claim 1.
 19. An expression vector comprising an expression cassette operably linked to the nucleic acid molecule of claim 18.
 20. The expression vector of claim 19, wherein the expression vector is an M13 phage-based plasmid.
 21. A host cell comprising the vector of claim 19.
 22. A method of preparing a fibronectin type III (Fn3) polypeptide monobody comprising the steps of:
 - a) providing a DNA sequence encoding a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein at least one loop region contains a unique restriction enzyme site, and wherein at least one of the plurality of Fn3 β -strand domain sequences are more stable at neutral pH than wild-type Fn3;
 - b) cleaving the DNA sequence at the unique restriction site;
 - c) inserting into the restriction site a DNA segment known to encode a peptide capable of binding to a specific binding partner (SBP) or a transition state analog compound (TSAC) so as to yield a DNA molecule comprising the insertion and the DNA sequence of (a);

and

- d) expressing the DNA molecule so as to yield polypeptide monobody.

23. A method of preparing a fibronectin type III (Fn3) polypeptide monobody comprising the steps of:

- (a) providing a replicatable DNA sequence encoding a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein the nucleotide sequence of at least one loop region is known, and wherein at least one of the plurality of Fn3 β -strand domain sequences are more stable at neutral pH than wild-type Fn3;
- (b) preparing polymerase chain reaction (PCR) primers sufficiently complementary to the known loop sequence so as to be hybridizable under PCR conditions, wherein at least one of the primers contains a modified nucleic acid sequence to be inserted into the DNA;
- (c) performing polymerase chain reaction using the DNA sequence of (a) and the primers of (b);
- (d) annealing and extending the reaction products of (c) so as to yield a DNA product; and
- (e) expressing the polypeptide monobody encoded by the DNA product of (d).

24. A method of preparing a fibronectin type III (Fn3) polypeptide monobody comprising the steps of:

- a) providing a replicatable DNA sequence encoding a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein the nucleotide sequence of at least one loop region is known, and wherein at least one of the plurality of Fn3 β -strand domain sequences are more stable at neutral pH than wild-type Fn3;

- b) performing site-directed mutagenesis of at least one loop region so as to create a DNA sequence comprising an insertion mutation; and
- c) expressing the polypeptide monobody encoded by the DNA sequence comprising the insertion mutation.
25. A kit for performing the method of any one of claims 22-24, comprising a replicatable DNA encoding a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein at least one of the plurality of Fn3 β -strand domain sequences are more stable at neutral pH than wild-type Fn3.
26. A variegated nucleic acid library encoding Fn3 polypeptide monobodies comprising a plurality of nucleic acid species each comprising a plurality of loop regions, wherein the species encode a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein one or more of the loop region sequences vary by deletion, insertion or replacement of at least two amino acids from corresponding loop region sequences in wild-type Fn3; wherein the β -strand domain sequences of the monobody have at least a 50% total amino acid sequence homology to the corresponding amino acid sequences of β -strand domain sequences of the wild-type Fn3; and wherein the Fn3 is more stable at neutral pH than wild-type Fn
27. The variegated nucleic acid library of claim 26, wherein one or more of the loop regions encodes:
- an AB amino acid loop from residue 15 to 16 inclusive;
 - a BC amino acid loop from residue 22 to 30 inclusive;
 - a CD amino acid loop from residue 39 to 45 inclusive;
 - a DE amino acid loop from residue 51 to 55 inclusive;
 - an EF amino acid loop from residue 60 to 66 inclusive; and

- vi) an FG amino acid loop from residue 76 to 87 inclusive.
28. The variegated nucleic acid library of claim 26, wherein the loop region sequences vary from the wild-type Fn3 loop region sequences by the deletion or replacement of at least 2 amino acids.
29. The variegated nucleic acid library of claim 26, wherein the monobody loop region sequences vary from the wild-type Fn3 loop region sequences by the insertion of from 3 to 25 amino acids.
30. The variegated nucleic acid library of claim 26, wherein a variegated nucleic acid sequence comprising from 6 to 75 nucleic acid bases is inserted in any one of the loop regions of the species.
31. The variegated nucleic acid library of claim 26, wherein the variegated sequence is constructed so as to avoid one or more codons selected from the group consisting of those codons encoding cysteine or the stop codon.
32. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the BC loop.
33. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the DE loop.
34. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the FG loop.
35. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the AB loop.
36. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the CD loop.

Fn3
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Cont

100-200-300-400-500-600-700-800-900

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Cont*
- X*
37. The variegated nucleic acid library of claim 26, wherein the variegated nucleic acid sequence is located in the EF loop.
38. A peptide display library derived from the variegated nucleic acid library of claim 26.
39. A peptide display library of claim 38, wherein the peptide is displayed on the surface of a bacteriophage or virus. *- species*
40. A peptide display library of claim 39, wherein the bacteriophage is M13 or fd.
41. A method of identifying the amino acid sequence of a polypeptide molecule capable of binding to a specific binding partner (SBP) so as to form a polypeptide:SSP complex wherein the dissociation constant of the polypeptide:SBP complex is less than 10^{-6} moles/liter, comprising the steps of:
- providing a peptide display library according to claim 39;
 - contacting the peptide display library of (a) with an immobilized or separable SBP;
 - separating the peptide:SBP complexes from the free peptides,
 - causing the replication of the separated peptides of (c) so as to result in a new peptide display library distinguished from that in (a) by having a lowered diversity and by being enriched in displayed peptides capable of binding the SBP;
 - optionally repeating steps (b), (c), and (d) with the new library of (d); and
 - determining the nucleic acid sequence of the region encoding the displayed peptide of a species from (d) and deducing the peptide sequence capable of binding to the SBP.
42. A method of preparing a variegated nucleic acid library encoding Fn3
- X*

polypeptide monobodies having a plurality of nucleic acid species each comprising a plurality of loop regions, wherein the species encode a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein one or more of the loop region sequences vary by deletion, insertion or replacement of at least two amino acids from corresponding loop region sequences in wild-type Fn3, and wherein the β -strand domain sequences of the monobody have at least a 50% total amino acid sequence homology to the corresponding amino acid sequences of β -strand domain sequences of the wild-type Fn3, and wherein the Fn3 comprises a stabilizing mutation β -strand domain, comprising the steps of

- a) preparing an Fn3 polypeptide monobody having a predetermined sequence;
- b) contacting the polypeptide with a specific binding partner (SBP) so as to form a polypeptide:SSP complex wherein the dissociation constant of the polypeptide:SBP complex is less than 10^{-6} moles/liter;
- c) determining the binding structure of the polypeptide:SBP complex by nuclear magnetic resonance spectroscopy or X-ray crystallography; and
- d) preparing the variegated nucleic acid library, wherein the variegation is performed at positions in the nucleic acid sequence which, from the information provided in (c), result in one or more polypeptides with improved binding to the SBP.

43. A method of identifying the amino acid sequence of a polypeptide molecule capable of catalyzing a chemical reaction with a catalyzed rate constant, k_{cat} , and an uncatalyzed rate constant, k_{uncat} , such that the ratio of k_{cat}/k_{uncat} is greater than 10, comprising the steps of:
- a) providing a peptide display library according to claim 39;
- b) contacting the peptide display library of (a) with an immobilized or separable transition state analog compound (TSAC) representing the approximate molecular transition state of the chemical

reaction;

- c) separating the peptide:TSAC complexes from the free peptides;
- d) causing the replication of the separated peptides of (c) so as to result in a new peptide display library distinguished from that in (a) by having a lowered diversity and by being enriched in displayed peptides capable of binding the TSAC;
- e) optionally repeating steps (b), (c), and (d) with the new library of (d); and
- f) determining the nucleic acid sequence of the region encoding the displayed peptide of a species from (d) and hence deducing the peptide sequence.

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44. A method of preparing a variegated nucleic acid library encoding Fn3 polypeptide monobodies having a plurality of nucleic acid species each comprising a plurality of loop regions, wherein the species encode a plurality of Fn3 β -strand domain sequences that are linked to a plurality of loop region sequences, wherein one or more of the loop region sequences vary by deletion, insertion or replacement of at least two amino acids from corresponding loop region sequences in wild-type Fn3, and wherein the β -strand domain sequences of the monobody have at least a 50% total amino acid sequence homology to the corresponding amino acid sequences of β -strand domain sequences of the wild-type Fn3, and wherein the Fn3 comprises a stabilizing mutation β -strand domain, comprising the steps of
- a) preparing an Fn3 polypeptide monobody having a predetermined sequence, wherein the polypeptide is capable of catalyzing a chemical reaction with a catalyzed rate constant, k_{cat} , and an uncatalyzed rate constant, k_{uncat} , such that the ratio of k_{cat}/k_{uncat} is greater than 10;
 - b) contacting the polypeptide with an immobilized or separable transition state analog compound (TSAC) representing the approximate molecular transition state of the chemical reaction;
 - c) determining the binding structure of the polypeptide:TSAC

complex by nuclear magnetic resonance spectroscopy or X-ray crystallography; and

- d) preparing the variegated nucleic acid library, wherein the variegation is performed at positions in the nucleic acid sequence which, from the information provided in (c), result in one or more polypeptides with improved binding to or stabilization of the TSAC.

45. An isolated polypeptide identified by the method of claim 41.
46. An isolated polypeptide identified by the method of claim 43.
47. A kit for identifying the amino acid sequence of a polypeptide molecule capable of binding to a specific binding partner (SBP) so as to form a polypeptide:SSP complex wherein the dissociation constant of the the polypeptide:SBP complex is less than 10^{-6} moles/liter, comprising the peptide display library of claim 39.
48. A kit for identifying the amino acid sequence of a polypeptide molecule capable of catalyzing a chemical reaction with a catalyzed rate constant, k_{cat} , and an uncatalyzed rate constant, k_{uncat} , such that the ratio of k_{cat}/k_{uncat} is greater than 10, comprising the peptide display library of claim 39.
49. A polypeptide derived by using the kit of claim 47.
50. A polypeptide derived by using the kit of claim 48.